Function assay result

Experiment 1: Function of transcriptional cascade

Purpose :

Testing the properties of three transcriptional cascade circuit, which composed of different ribosome binding site and duplication of CI coding region respectively.

[Notice]

Before reading further details, please notice that we have named these circuits with three symbols:

II*2: pfadBA-RBS34-CI-pfadBA-RBS34-CI-pCI-RBS34-GFP40-term

II 34: pfadBA-RBS34-CI-pCI-RBS34-GFP40-term

II 30: pfadBA-RBS30-CI-pCI-RBS34-GFP40-term

Method :

1. Three E.coli strains is cultured overnight in 200mL LB medium respectively in Erlenmeyer flask, under 37℃ environment.
2. 10mL oleic acid is added to the flasks respectively and mixed by hand-shake for 1 minute.
3. 30ml LB-bacteria mixture is moved from the flasks every 30 minutes. Then the lysis buffer is added to the mixture.

100 c.c Lysis buffer:

$Na_3PO_4 \cdot 12H_2O$	1.9006 g
NaCl	0.5844 g
EDTA(0.5M)	20λ
Triton X 100	200λ

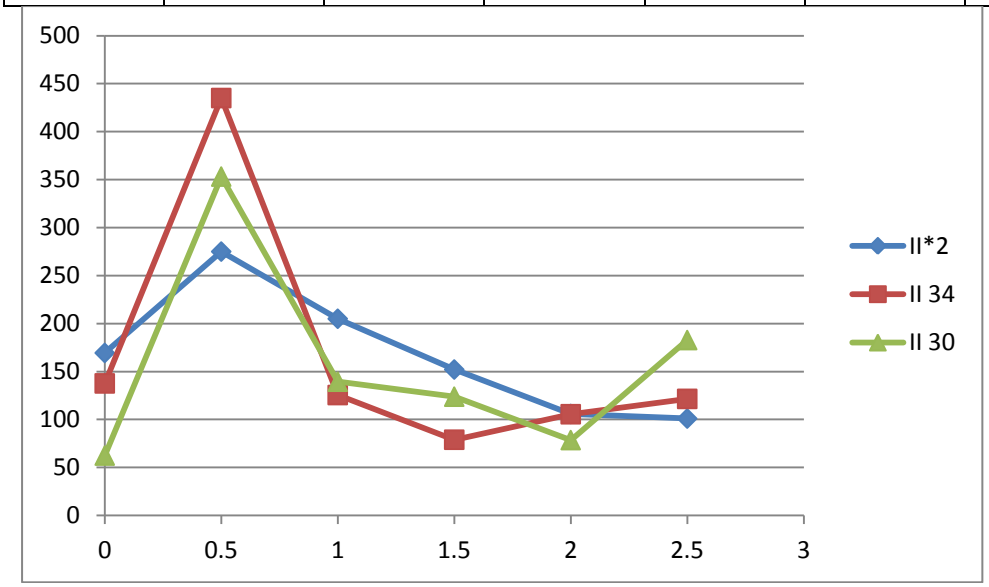
Add 1/1000 V β-mercaptoethanol & 120λ protein inhibitor/c.c(Roche)

4. E.coli in LB medium with lysis buffer is destructed by sonicator.
5. Centrifuge by 9000 rpm, 15 minutes.
6. Move the supernatant liquid into eppendorfs.
7. Records the absorbance of green fluorescence protein with fluorophotometer.

Result :

hour	0	0.5	1	1.5	2	2.5

II*2	169.4	275	205.0	152.1	105.8	101
II 34	137.6	435	125.5	78.91	105.5	121.5
II 30	62.44	353.2	139.4	123.8	78.55	182.8



Experiment 2: Function of J23119-RBS34-FadR-terminator-J23119-RBS34-FadL-terminator

Purpose:

Confirm that FadR protein and FadL protein assist the bacterial uptake of fatty acid.

Method:

1. We use modified LB medium for culturing E.coli strain. The amount of yeast extract is decreased.

10 g tryptone

1 g yeast extract

10 g NaCl /1L ddH2O

We added 20λ, 40λ, 60λ, 80λ, 100λ oleic acid in 2mL modified LB medium respectively. This fatty acid – composing broth is used for culturing E.coli.

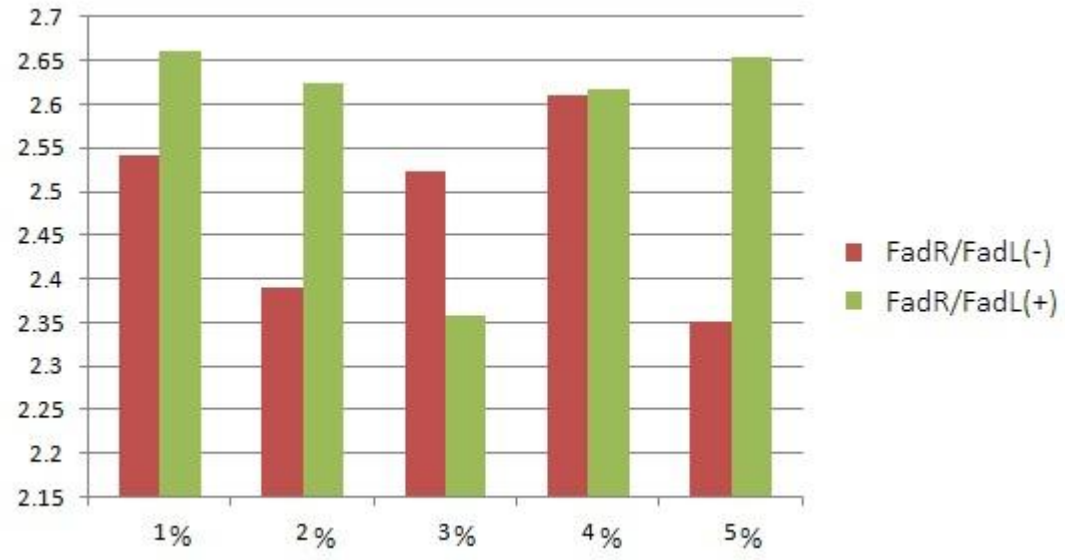
2. The E.coli strain with FadR/FadL coding gene and the DH5α cell (without FadR/FadL coding gene) is cultured overnight in the modified LB medium, 2 c.c respectively, with different fatty acid ratio.

3. Record the absorbance of the suspension culture by spectrophotometer.

Since the amount of yeast extract is decreased significantly in LB medium, the bacteria is forced to metabolizes oleic acid for carbon source (yeast extract is the main carbon source in origin LB medium). Therefore, the E.coli strain that contain FadR/FadL coding gene will be more adjustable to the critical environment and grow better in the medium. We compared the growing condition of FadR/FadL (+) and FadR/FadL (-) strain in modified medium

Result:

V% of oleic acid	plasmid (-)	plasmid(+)
1%	2.542	2.661
2%	2.390	2.623
3%	2.523	2.357
4%	2.610	2.616
5%	2.351	2.653



FadR/FadL(+) E.coli strain grows better in the critical environment, compared to the control group.